



# **Authorizations and Permits for Protected Species (APPS)**

File #: 14506

Title: Population structures, relative abundance, ha

Modification: 1

### **Applicant Information**

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### **Project Information**

File Number: 14506

Application Status:

**Application Complete** 

Project Title: Population structures, relative abundance, habitat utilization, physiology, epidemiology, and genetics of juvenile marine turtles on the Atlantic coast of Florida.

Project Status: Renewal

Previous

Federal or

State Permit: 1507

Permit • ESA Section 10(a)(1)(A) permit (other)

Requested:

Where will

US Locations including offshore waters

activities occur?

Research

Timeframe: Start: 09/03/2010 End: 09/15/2015

Sampling

Project 1: Central Region of the Indian River Lagoon, Florida - Year-round for Five Years This project started in 1982 and is intended to continue indefinitely.

Season/Project Duration:

Project 2: Sabellariid Worm Rock Reefs in the Near-shore Waters of Indian River County, Florida - Late Spring and Summer Months for Five Years This project started in 1989

and is intended to continue indefinitely.

Project 3: Trident Turning Basin, Cape Canaveral Air Force Station, Cape Canaveral, Florida - Seasonally to the extent Navy operations will allow for Five Years. It should be noted that the Trident Basin is a high security area and the Navy schedule of operations can change literally at the last minute and for extended periods of time. This project began

in 1993 and is intended to continue indefinitely.

Abstract:

This permit application is to continue long-term projects studying the juvenile marine turtle populations in three disparate developmental habitats on Florida's Atlantic coast. Project 1: The central region of the Indian River Lagoon System, Project 2: The Sabellariid worm rock reefs in the near-shore waters of Indian River County, Project 3: The Trident Turning Basin, Cape Canaveral Air Force Station. Target Species: loggerhead turtle (Caretta caretta), green turtle (Chelonia mydas), Kemp's ridley turtle (Lepidochelys kempii), hawksbill turtle (Eretmochelys imbricata), leatherback turtle (Dermochelys coriacea). Marine turtles are captured using tangle nets and dip nets to assess the population structure, trends in relative abundance, habitat utilization, sex ratios, physiology, genetics, zoogeography, and epidemiology of marine turtles in these habitats. Project 1 Annual Take: Capture, flipper tag, PIT tag, measure, weigh, blood sample, tissue biopsy, lavage, photograph/yr C. caretta 10, C. mydas 250, L. kempii 3, E. imbricata 2, D. coriacea 1; attach satellite tag/yr C. mydas 10. Project 2 Take: Capture, flipper tag, PIT tag, measure, weigh, blood sample, tissue biopsy, lavage, photograph/yr C. caretta 10, C. mydas 140, L. kempii 2, E. imbricata 2. Project 3 Take: Capture, flipper tag, PIT tag, measure, weigh, blood sample, tissue biopsy, lavage, photograph/yr C. caretta 10, C. mydas 140, L. kempii 1, E. imbricata 1, D. coriacea 1. These projects will also provide researchers from other institutions and governmental agencies, independently permitted by NMFS, access to marine turtles. This is an application for a 5 year permit.

### **Project Description**

Purpose:

Project 1: Central Region of the Indian River Lagoon System, Florida

The objectives of this project are to: (1) Continue to monitor the structure of populations and trends in the relative abundance of marine turtles utilizing the central region of the Indian River Lagoon as habitat. (2) collect blood samples and tissue biopsies for epidemiological studies of diseases afflicting marine turtles. (3) Collect blood samples to monitor the sex ratios in these immature marine turtle populations. (4) Obtain tissue biopsies for stable isotope analysis. (5) Continue to otain lavage samples from green turtles for feeding ecology studies which will monitor changes in their diet at our current study site (the abundance of macroalgae species are known to change over time). As funding becomes available our study will be expanded to other areas of the central region of the Indian River Lagoon system and diet analysis will be an essential activity. (6) Collect blood samples for fibropapillomatosis, genetic, and sex ratio research. Tissue biopsies are taken for genetic analysis when blood samples can not be obtained. (7) Attach satellite transmitters to track the movements of immature marine turtles within this developmental habitat and between the central region of the Indian River Lagoon and other developmental or adult foraging habitats in the Atlantic Ocean, Gulf of Mexico, and Caribbean Sea.

This project was initiated in 1982. It has shown that the study area in the central region of the Indian River Lagoon serves as a developmental habitat for both loggerheads and green turtles (Ehrhart et al., 2007). Also, Kemp's ridleys, hawksbills, and leatherbacks have either been captured or observed there (ibid). The Recovery Plan for the U.S. Population of Atlantic Green Turtle (1991) states that the foremost problem in management and conservation of sea turtles is the lack of basic biological information. Included in the step down plan is the need to determine green turtle distribution, abundance and status in the marine environment. The Recovery Plan for the Northwest Atlantic Population of Loggerhead Turtle (Caretta caretta), Second Revision (2008) cites the need to determine demographic parameters, refine population genetic structure, and monitor distribution, abundance, and trends. Results of work proposed here will continue to provide valuable information necessary for wildlife managers and agencies on the state and federal levels to make management decisions regarding recovery and conservation of green turtles, hawksbill turtles, Kemp's ridley turtles, and leatherback turtles considered as endangered under the Endangered Species Act, and loggerhead turtles, considered threatened under the Endangered Species Act. It is important to note that this is the only long-term study of its kind on the east coast of the United States as it has been ongoing for 27 years and one of the few world-wide that has been ongoing for such an extended period of time. The data obtained by achieving the objectives outlined above will contribute to the understanding of (a) the demographics of these populations, (b) the attributes of the Indian River Lagoon that make it a preferred developmental habitat for these species, (c) the heretofore poorly understood movements of immature marine turtles between developmental habitats and their eventual movement to adult foraging habitats, by use of satellite tagging and flipper taggin

#### Project 2: Sabellariid Worm Rock Reefs in the Near-shore Waters of Indian River County, Florida

The objectives of this project are to: (1) Continue to monitor the structure and trend in relative abundance of marine turtle populations utilizing the Sabellariid worm rock reef habitat. (2) Collect blood samples and tissue biopsies for epidemiological studies of diseases afflicting green turtles. (3) Collect blood samples to monitor the sex ratios of immature marine turtle populations which may change over time due to climate change. (4) Obtain tissue biopsies for stable isotope analysis. (5) Collect lavage samples for feeding ecology studies to monitor changes in diet over time. (6) Collect blood samples and tissue biopsies for genetic analysis to determine changes in the source rookeries of green turtles in this habitat. This project was initiated in 1989. It has shown that the study area in the nearshore waters of northern Indian River County serves as a developmental habitat for green turtles but only marginally for loggerheads. Two hawksbills been captured there. The Recovery Plan for the U.S. Population of Atlantic Green Turtle (1991) states that the foremost problem in management and conservation of sea turtles is the lack of basic biological information. Included in the step down plan is the need to determine green turtle distribution, abundance and status in the marine environment. Results of work proposed here will continue to provide valuable information necessary for wildlife managers and agencies on the state and federal levels to make management decisions regarding recovery and conservation of green turtles, considered as endangered under the Endangered Species Act. It is important to note that this is the only long-term study of its kind on the east coast of the United States. The data obtained by achieving the objectives outlined above will contribute to the understanding of (a) the demographics of this population, (b) the attributes of the Sabellariid worm rock reefs that make it a preferred developmental habitat for this species, and (c) the epidemiology of diseases afflicting marine turtles

#### Project 3: Trident Turning Basin, Cape Canaveral Air Force Station, Cape Canaveral, Florida

The objectives of this project are to (1) continue to monitor the structure and trend in relative abundance of marine turtle populations utilizing the Trident Turning Basin habitat, (2) collect blood samples and tissue biopsies for epidemiological studies of diseases afflicting green turtles, (3) collect blood samples to monitor the sex ratios of immature marine turtle populations, (4) obtain tissue biopsies for stable isotope analysis and lavage samples for feeding ecology studies, and (5) collect blood samples and tissue biopsies for genetic analysis to determine the source rookeries of the marine turtles in this habitat.

This project was initiated in 1993. It has shown that the Trident Turning Basin serves as a developmental habitat for green turtles almost exclusively (Redfoot, 1997). The Recovery Plan for the U.S. Population of Atlantic Green Turtle (1991) states that the foremost problem in management and conservation of sea turtles is the lack of basic biological information. Included in the step down plan is the need to determine green turtle distribution, abundance and status in the marine environment. Results of work proposed here will continue to provide valuable information necessary for wildlife managers and agencies on the state and federal levels to make management decisions regarding recovery and conservation of green turtles, considered as endangered under the Endangered Species Act. The data obtained by achieving the objectives outlined above will contribute to the

understanding of (a) the demographics of this population, (b) the attributes of the Trident Turning Basin that make it a preferred developmental habitat for this species, and (c) the epidemiology of diseases afflicting marine turtles.

For all three projects the overall purpose of this research is to elucidate patterns and trends in the abundance, distribution, and population structure of these threatened and endangered species. We are also gaining a greater understanding of the attributes of the habitats utilized by these species, the anthropomorphic threats they face, and the zoogeography of these animals. The use of alternative species is not an option. We endeavor to obtain as many data as possible from each animal captured.

Description: Project 1: Central Region of the Indian River Lagoon System, Florida

Turtles are captured by large-mesh tangle nets, constantly attended, deployed for up to six hours in the central region of the Indian River Lagoon System on Florida's east coast. The nets consist of webbing hung from a braided polypropylene top line (0.635 cm in diameter) that is suspended at the surface by floats attached during deployment. The webbing is made of 18 ga. twisted nylon twine, with a 40 cm knot to knot stretch mesh. The bottom line of each net is made of No. 30 continuous lead core line. The nets are 3.7 m deep and approximately 230 m in length set in water 2.5 meters deep. Normally two nets are tied end-to-end and deployed. Net deployment begins with the rigging of an 8 kg Danforth-type anchor. A 1.5 m section of 0.8 cm chain is shackled to the ring on the anchor shaft. Another shackle is used to attach a 15 m length of 1 cm nylon line to the chain. The other end of the line is tied to the free end of the top line of the first net. The nets are paid out from the uncovered bow of a 17 ft Boston Whaler boat operated in reverse. The bow is free of all cleats and other hardware that would interfere with the deployment, tending and retrieval of the net. The anchor is lowered to the bottom as the boat moves away and the entire length of anchor line is paid out. At that point it is tested to assure that the anchor has penetrated the bottom and is holding. That having been done, a float is attached to the top line at the point where the webbing begins and the net mesh begins to enter the water. Two or three workers tend the net as it is paid out, making sure that the bottom line does not get twisted over the top line and attaching floats at 10 m intervals. The time at which the mesh first begins to enter the water and at which the last of the mesh is soaked is recorded. A second anchor, rigged in the manner described above, is tied to the free end of the top line of the second net when the last of the webbing is deployed. A worker holds onto the second anchor until the line is taut and then lowers it overboard. Net deployment always begins at the upwind end of the netting site and the boat operator sets a course that is at about a 45-degree angle to the wind. This assures that the wind will keep the stern and propeller away from the net during subsequent net tending. Once deployed the net is continuously tended by personnel elevating the top line of the net from the bow of a Boston Whaler boat. Two boats are used; as the personnel in one boat reach the mid-point of the net, the personnel in the second boat start checking at the head of the net. This ensures that any given point along the net is checked by the elevation of the top line approximately every ten minutes. Additionally, personnel on both boats are in continuous visual contact with the net. Large-hoop dip nets are used to aid in the capture and boarding of entangled turtles. Captured turtles are transferred to a third boat where they are tagged, measured, weighed, a blood and/or tissue sample collected, and photographed. The turtles are released at or within a short distance of the capture location within a few hours on the same day.

Captured turtles are flipper tagged with inconel metal tags (cattle ear tag #681, manufactured by National Band and Tag Company) on a scale proximal to the body on the trailing edge of each front flipper, and a PIT tag is inserted subcutaneously in the right front flipper. The application and antiseptic protocol described in Research and Management Techniques for the Conservation of Sea Turtles (Eckert, et al., 1999) are used. The inconel tag applicators are cleaned with a mild bleach solution before use on each turtle and a separate set of applicators is used with turtles afflicted with fibropapillomatosis. Prepackaged sterile PIT tags are used and the site of injection wiped with alcohol swabs both before and after insertion. Tag loss for inconel tags is expected but impossible to predict how long they will stay in place. We have been inserting P.I.T. tags since the early 1990s and to the best of our knowledge have only lost one. Turtles are detained only long enough for data collection to be completed.

Measurements of straight carapace length (standard carapace length), maximum straight carapace length, straight carapace width, head width, and body depth are made with forestry calipers. Curved carapace length, curved carapace width, plastron length, and tail length measurements are made with a cloth tape. Weight is obtained with a spring scale. All measurements are made using the protocol described by Bolten (1999). The calipers and tape are cleaned with a mild bleach solution before each turtle is measured. A separate set of calipers and tapes are used for turtles afflicted with fibropapillomatosis.

Blood is drawn using antiseptic protocol from the dorsal cervical sinus (Owens, 1999) of each turtle for genetic analysis to estimate population origins, for epidemiological research, and for sex determination.

Tissue biopsys are performed using the protocol described by Dutton and Balazas (1995). The area to be biopsied is first scrubbed with an isopropyl alcohol swab. The tissue biopsy is

obtained using a 4mm sterile biopsy punch. If needed, a coagulant powder is used to control any excessive bleeding afterwards.

Epibionts (leeches) are removed using forceps. The removal site is then swabbed with either isopropyl alcohol or betadine.

Samples of food item consumed by juvenile green turtles are obtained by a lavage of the esophagus using a modification of the methods described by Legler (1977), Balazs (1980), and Forbes and Limpus (1993). Each turtle is turned on its back with its posterior slightly elevated. A soft plastic veterinarian's stomach tube, lubricated with vegetable oil, is carefully inserted through the mouth and down the length of the esophagus. A 9 mm outside diameter (OD), 6 mm inside diameter (ID) tube is used with turtles in the 20 cm to 35 cm SCL size classes; a somewhat larger tube, 13 mm OD and 8 mm ID tube is used with turtles in size classes larger than 35 cm SCL. A moderate volume of water is pumped through the tube using a veterinarian's double action stomach pump as the tube is gently moved up and down the length of the esophagus. Turtles are lavaged for no more than 45 seconds and generally for less than 30 seconds. The lavage procedure was approved by Lawrence Herbst DVM, Ph.D. (Albert Einstein College of Medicine, Bronx, NY), and George Balazs (National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Laboratory, Honolulu, HI), a marine turtle researcher experienced with lavage. Only one sample is obtained per individual.

The Fast-lock GPS/Argos satellite tags manufactured by Wildlife Computers, Richmond Washington, are 10.2 cm long, 5.7 cm wide, 3.1 cm high (not including the antenna), and weigh 225 g. The anterior portion of the carapace is cleaned of sediment and algae. Coarse sandpaper is used to scuff up the first, second, and part of the third vertebral plus the first and second costal scutes on both sides. Care is taken to avoid the seams between scutes. The satellite tag is attached to the turtle using both Sonic-Weld, an epoxy putty, and Power-Fast, a two-part epoxy that cures releasing little heat. The attachment process takes approximately two hours. The turtle is then released close to where it was captured. All the procedures describe above are performed by or under the supervision of the principal investigator (PI) or one of the co-investigators (CI). No injury or mortality of marine turtles has resulted from any of the procedures described above.

L.M. Ehrhart, the PI, has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 35 years. He has been obtaining blood samples, lavaging, and obtaining biopsy samples for 20 years. He has been attaching satellite tags for 10 years. W.E. Redfoot, a CI, has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 27 years. He has been attaching satellite tags for 10 years. D.A. Bagley, a CI, has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 21 years. She has been obtaining blood samples, lavaging, and obtaining biopsy samples for 21 years. She has been attaching satellite tags for 10 years. S.A. Ceriani has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 2 years. She has been obtaining blood samples, lavaging, and obtaining biopsy samples for 2 years. She has been attaching satellite tags for 2 years. B. Shamblin has at least 3 years experience with marine turtles.

No drugs are administered to the turtles.

It is anticipated that as many as 250 green turtles (Chelonia mydas) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, lavaged, photographed, and released annually. Ten juvenile green turtles between 40 and 60 cm SCL will be captured, flipper tagged, PIT tagged, measured, photographed, satellite transmitter attached, and released annually.

It is anticipated that as many as 100 loggerhead turtles (Caretta caretta) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. It is anticipated that as many as 3 Kemp's ridley turtles (Lepidochelys kempii) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. It is anticipated that as many as 2 hawksbill turtles (Eretmochelys imbricata) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. It is anticipated that 1 leatherback turtle (Dermochelys coriacea) will be captured, flipper tagged, PIT tagged, measured, blood sampled, tissue biopsies obtained, photographed, and released annually. It is very difficult to precisely list the number of turtles of each species that may be captured and processed because of variations in netting conditions and naturally occurring fluctuations in populations. Although the anticipated capture rates listed above may seem inflated, they are listed as such to cover possible eventualities and prevent disruptions in contract commitments.

### Project 2: Sabellariid Worm Rock Reefs in the Near-shore Waters of Indian River County, Florida

As with the lagoon project, a large mesh tangle net anchored at each end is used to capture turtles. The net is 3.7 m deep, has a 40 cm stretch (knot to knot) mesh size, and is 220 m long. It is hung from a braided polypropylene top line that is suspended at the surface by floats attached at intervals during deployment. The bottom line is made of No. 30 continuous

lead core line. The net is set over the sandy corridors between reefs in water that is 2 to 3.5 m deep. Elevating the net from the bow of a boat is impractical in the near-shore ocean conditions. Instead, relays of six swimmers, working in pairs, equipped with mask, snorkel, and fins continuously patrol its length. Every two to four minutes any given point along the net is monitored by a pari of swimmers. Within moments after a turtle becomes entangled, a swimmer dives down and brings the turtle to the surface where it is disentangled. Two boats are used in the netting operation, a 19-foot Boston Whaler and either a 17-foot or a 15-foot Boston Whaler. One is used to deploy and retrieve the net (the 19') and both are used to pick up captured turtles and serve as rest stations for swimmers. Captured turtles are covered with wet towels and kept aboard the boats. If a turtle has symptoms of fibropapillomatosis it is segregated from any other captures. The turtles are worked up and released at the site of capture.

It should be noted that the principal investigator pioneered the use of tangle nets for the capture of marine turtles over the Sabellariid worm-rock reef developmental habitat, and is the lead co-author of the chapter "Studies in Foraging Habitats: Capturing and Handling Turtles" in Research and Management Techniques for the Conservation of Sea Turtles (Eckert et al., 1999).

Captured turtles are flipper tagged with inconel metal tags (cattle ear tag #681, manufactured by National Band and Tag Company) on a scale proximal to the body on the trailing edge of each front flipper, and a PIT tag is inserted subcutaneously in the right front flipper. The application and antiseptic protocol described in Research and Management Techniques for the Conservation of Sea Turtles (ibid) are used. The inconel tag applicators are cleaned with a mild bleach solution before use on each turtle and a separate set of applicators is used with turtles afflicted with fibropapillomatosis. Prepackaged sterile PIT tags are used and the site of injection wiped with alcohol swabs both before and after insertion. Tag loss for inconel tags is expected but impossible to predict how long they will stay in place. We have been inserting P.I.T. tags since the early 1990s and to the best of our knowledge have only lost one.

No drugs are used. Turtles are retained only long enough for data collection to be completed. Turtles afflicted with fibropapillomatosis are kept separate from those not displaying symptoms. A separate set of measuring and tagging equipment is used for infected animals.

Measurements of straight carapace length (standard carapace length), maximum straight carapace length, straight carapace width, head width, and body depth are made with forestry calipers. Curved carapace length, curved carapace width, plastron length, and tail length measurements are made with a cloth tape. Weight is obtained with a spring scale. All measurements are made using the protocol described by Bolten (1999). The calipers and tape are cleaned with a mild bleach solution before each turtle is measured. A separate set of calipers and tapes are used for turtles afflicted with fibropapillomatosis.

Blood is drawn using antiseptic protocol from the dorsal cervical sinus (Owens, 1999) of each turtle for genetic analysis to estimate population origins, for epidemiological research, and for sex determination. Tissue biopsys are performed using the protocol described by Dutton and Balazas (1995). The area to be biopsied is first scrubbed with an isopropyl alcohol swab. The tissue biopsy is obtained using a 4mm sterile biopsy punch. If needed, a coagulant powder is used to control any excessive bleeding afterwards. Epibionts (leeches) are removed using forceps. The removal site is then swabbed with either isopropyl alcohol or betadine.

Samples of food item consumed by the turtles are obtained by a lavage of the esophagus using a modification of the methods described by Legler (1977), Balazs (1980), and Forbes and Limpus (1993). Each turtle is turned on its back with its posterior slightly elevated. A soft plastic veterinarian's stomach tube, lubricated with vegetable oil, is carefully inserted through the mouth and down the length of the esophagus. A 9 mm outside diameter (OD), 6 mm inside diameter (ID) tube is used with turtles in the 20 cm to 35 cm SCL size classes; a somewhat larger tube, 13 mm OD and 8 mm ID tube is used with turtles in size classes larger than 35 cm SCL. A moderate volume of water is pumped through the tube using a veterinarian's double action stomach pump as the tube is gently moved up and down the length of the esophagus. Turtles are lavaged for no more than 45 seconds and generally for less than 30 seconds. The lavage procedure was approved by Lawrence Herbst DVM, Ph.D. (Albert Einstein College of Medicine, Bronx, NY), and George Balazs (National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Laboratory, Honolulu, HI), a marine turtle researcher experienced with lavage. Only one sample is obtained per individual. No injury or mortality of marine turtles has resulted from any of the procedures described above.

All the procedures describe above are performed by or under the supervision of the principal investigator (PI) or one of the co-investigators (CI). L.M. Ehrhart, the PI, has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 35 years. He has been obtaining blood samples, lavaging, and obtaining biopsy samples for 20 years. W.E. Redfoot, a CI, has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 27 years. He has been obtaining blood samples, lavaging, and obtaining blood samples for 20 years. D.A. Bagley, a CI, has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 21 years. She has been obtaining blood samples, lavaging, and obtaining biopsy samples for 21 years. S.A. Ceriani has been capturing using tangle nets, measuring, weighing, and tagging marine turtles for 2 years. She has

been obtaining blood samples, lavaging, and obtaining biopsy samples for 2 years. B. Shamblin has at least 3 years experience with marine turtles.

It is anticipated that 140 green turtles (Chelonia mydas) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, lavaged, photographed, and released annually. It is anticipated that 10 loggerhead turtles (Caretta caretta) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. It is anticipated that 2 kemp's ridley turtles (Lepidochelys kempii) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. It is anticipated that 2 hawksbill turtles (Eretmochelys imbricata) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. Although these take figures may seem exaggerated when compared to the number of captures over the past five years, there have been some years when as many as 220 green turtles were caught in one season. There were several years when the number of captures exceeded 100. Over the past five years weather conditions and poor water clarity have severely restricted our ability to work in this study area. Again, although the anticipated capture rates listed above may seem inflated, they are listed as such to cover possible eventualities and prevent disruptions in contract commitments.

#### Project 3: Trident Turning Basin, Cape Canaveral Air Force Station, Cape Canaveral, Florida

The Trident Turning Basin encompasses approximately one square kilometer. The structure of the green turtle population is monitored by capturing individuals using both tangle nets and large dip nets. The tangle nets consist of nylon twine mesh hung from a braided polypropylene top line and a No. 30 continuous lead core bottom line. Two tangle nets are used, set individually. One is 238 m in length, 3.7 m in depth with a 40 cm stretch mesh size (knot to knot). The other is 229 m in length, 3.7 m in depth, and has a 30.5 cm stretch mesh size.

The nets are deployed at various locations along the walls over the shallow shelf on the perimeter of the basin. The nets are suspended from floats attached at regular intervals to the top line during deployment. Each net is checked on a regular basis by elevating the top line from the bow of a small boat. Any given portion of a tangle net is checked approximately every 15 minutes by pulling hand over hand along the top line from the bow of a boat. Turtles are also opportunistically captured with long handled, large-hoop dip nets. Following their capture, the turtles are transported to shore, where they are flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, lavaged, and photographed.

To identify turtles in the follow-up population estimation survey (described below), a unique number is painted on the carapace of all captured turtles using non-toxic white paint. All turtles are released back into the basin not more than 8 hours of their capture and generally within 6 hours. They are not released immediately after being processed to avoid their recapture that day and to facilitate carapace painting.

Captured turtles are flipper tagged with inconel metal tags (cattle ear tag #681, manufactured by National Band and Tag Company) on a scale proximal to the body on the trailing edge of each front flipper on each front flipper, and a PIT tag is inserted subcutaneously in the right front flipper. The application and antiseptic protocol described in Research and Management Techniques for the Conservation of Sea Turtles (Eckert, et al.) are used. The inconel tag applicators are cleaned with a mild bleach solution before use on each turtle and a separate set of applicators is used with turtles afflicted with fibropapillomatosis. Prepackaged sterile PIT tags are used and the site of injection wiped with alcohol swabs both before and after insertion. Tag loss for inconel tags is expected but impossible to predict how long they will stay in place. We have been inserting P.I.T. tags since the early 1990s and to the best of our knowledge have only lost one

No drugs are used.

The turtles are held in large plastic mason's tubs in water and covered with moist towels during the time of data collection until they are released. Turtles with fibropapillomatosis are kept in separate tubs from turtles without fibropapillomatosis. The tubs are disinfected after their use. A separate set of measuring and tagging equipment is used for infected animals, or the equipment used is disinfected with a bleach solution prior to use with non-infected animals.

Measurements of straight carapace length (standard carapace length), maximum straight carapace length, straight carapace width, head width, and body depth are made with forestry calipers. Curved carapace length, curved carapace width, plastron length, and tail length measurements are made with a cloth tape. Weight is obtained with a spring scale. All measurements are made using the protocol described by Bolten (1999). The calipers and tape are cleaned with a mild bleach solution before each turtle is measured. If a turtle afflicted with fibropapillomatosis is captured in the Trident Basin a separate set of calipers and tapes will be used.

To estimate the population size of juvenile green turtles in the Trident Basin using a modification of the Lincoln-Petersen mark and recapture method for closed populations. A

unique number is painted on the carapace of each captured turtle using a non-toxic white spray paint which wears off within a few days. One or two days after the capture session, observers in a small boat slowly travel around the edge of the basin recording observations of painted and unpainted green turtles. Observed painted, i.e., marked, turtles are counted as "recaptures" and unpainted as new captures. No turtles are actually captured. A population estimate is calculated from these observations. Loggerheads are rarely captured in the Trident Basin and are not part of the population estimation, therefore are not painted.

Blood is drawn using antiseptic protocol from the dorsal cervical sinus (Owens, 1999) of each turtle for genetic analysis to estimate population origins, for epidemiological research, and for sex determination.

Food item samples are obtained from turtles by a lavage of the esophagus using a modification of the methods described by Legler (1977), Balazs (1980), and Forbes and Limpus (1993). Each turtle is turned on its back with its posterior slightly elevated. A soft plastic veterinarian's stomach tube, lubricated with vegetable oil, is carefully inserted through the mouth and down the length of the esophagus. A 9 mm outside diameter (OD), 6 mm inside diameter (ID) tube is used with turtles in the 20 cm to 35 cm SCL size classes; a somewhat larger tube, 13 mm OD and 8 mm ID tube is used with turtles in size classes larger than 35 cm SCL. A moderate volume of water is pumped through the tube using a veterinarian's double action stomach pump as the tube is gently moved up and down the length of the esophagus. Turtles are lavaged for no more than 45 seconds and generally for less than 30 seconds. The lavage procedure was approved by Lawrence Herbst DVM, Ph.D. (Albert Einstein College of Medicine, Bronx, NY), and George Balazs (National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Laboratory, Honolulu, HI), a marine turtle researcher experienced with lavage. Only one sample is obtained per individual. Injury or mortality of marine turtles has resulted from the procedures described above.

Epibionts (leeches) are removed using forceps. The removal site is then swabbed with either isopropyl alcohol or betadine.

Tissue biopsys are performed using the protocol described by Dutton and Balazas (1995). The area to be biopsied is first scrubbed with an isopropyl alcohol swab. The tissue biopsy is obtained using a 4mm sterile biopsy punch. If needed, a coagulant powder is used to control any excessive bleeding afterwards.

All the procedures describe above are performed by or under the supervision of the principal investigator (PI) or one of the co-investigators (CI) whose qualifications are noted above for projects one and two.

It is anticipated that 140 green turtles (Chelonia mydas) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, lavaged, photographed, and released annually. It is anticipated that 10 loggerhead turtles (Caretta caretta) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, and released annually. It is anticipated that 1 Kemp's ridley turtle (Lepidochelys kempii) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. It is anticipated that 1 hawksbill turtles (Eretmochelys imbricata) will be captured, flipper tagged, PIT tagged, measured, weighed, blood sampled, tissue biopsies obtained, photographed, and released annually. Although the anticipated capture rates listed above may seem inflated, they are listed as such to cover possible eventualities and prevent disruptions in contract commitments. It should be noted that from September 2008 to August 2009 126 juvenile green turtles and 3 loggerheads were captured in the Trident Basin.

### **Supplemental Information**

Status of Species: Green Turtle (Chelonia mydas) endangered

Hawksbill (Eretmochelys imbricata) endangred Kemp's Ridley (Lepidochelys kempii) endangred

Loggerhead (Caretta caretta) threatened

Leatherback Turtle (Dermochelys coriacea) endangered

Lethal Take: Not Applicable

Anticipated Effects on Animals:	Any time a turtle is removed from its natural habitat and handled, it undoubtably experiences stress. However, based on our observations over decades of research, our procedures have had minor, if any, adverse effects on the captured turtles. No marine mammals have ever been captured. Sharks and rays are captured fairly frequently but are quickly untangled and released. Every few years a large boney fish is captured, but is quickly untangled and released.
Measures to Minimize Effects to Listed Species:	Turtles are covered with wet towels while being held, disinfection of tagging equipment, disinfection of holding areas and tubs, antiseptic protocol followed when drawing blood or taking biopsies, and the turtles are released as soon as possible.
Resources Needed to Accomplish Objectives:	L. M. Ehrhart, Ph.D. has over thirty-three years of in-water sea turtle research experience. W. E. Redfoot, M.S. has over 27 years of in-water sea turtle research experience. D. A. Bagley, M.S. has over 21 years of in-water sea turtle research experience. S. A. Ceriani has over 5 years of sea turtle research, at least two of them in-water. L. M. Ehrhart is Professor Emeritus, and W. E. Redfoot and D. A. Bagley are research associates in the Department of Biology, University of Central Florida, Orlando, Florida. S. A. Ceriani is a Ph.D student at the University of Central Florida supplies boats, tow vehicles, nets, and other equipment required to achieve research objectives.
Disposition of Tissues:	All blood and tissue samples are processed at the University of Central Florida by qualified, permitted personnel and are consumed in analysis. They are stored at the university or at a facility made available to us by the U.S. Fish and Wildlife Service in south Brevard County, Florida. If samples are collected for qualified, permitted individuals at other institutions or agencies, those individuals will supply required information before any samples are transfered.
Public Availability of Product/Publications:	All reports or copies of publications will be supplied on request

# **Location/Take Information**

# Location

Research Area: Atlantic Ocean State: FL Stream Name: Indian River Lagoon System, Central Region Location Description: The study area ranges from 28.2 Latitude, -80.6 Longitude to 27.5 Latitude, -80.3 longitude. Included is the study area is the Pelican Island Wildlife Refuge.

### **Take Information**

		Listing	Production	•		Expected	Takes Per		Observe /Collect		Transport	Begin	
Line Ve	Species	Unit/Stock	/Origin	Life Stage	Sex	Take	Animal	Take Action	Method	Procedure	Record	<b>Date</b>	<b>End Date</b>
1	Turtle, green sea	Range-wide (NMFS Threatened)	Wild	Adult/ Subadult/ Juvenile	Male and Female	250	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Lavage; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015

2	Turtle, loggerhead sea	Range-wide (NMFS Threatened)	Wild	Adult/ Subadult/ Juvenile	Male and Female	100	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010 9/15/2015
3	Turtle, green sea	Range-wide (NMFS Threatened)	Wild	Juvenile/ Subadult	Unknown	10	1	Capture/Handle/Release	Net, Tangle	Instrument, epoxy attachment (e.g., satellite tag, VHF tag); Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Tracking; Weigh	N/A	9/3/2010 9/15/2015
5	Turtle, Kemp's ridley sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Unknown	3	1	Capture/Handle/Release	Net, Tangle	Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010 9/15/2015
6	Turtle, hawksbill sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Unknown	2	1	Capture/Handle/Release	Net, Tangle	Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010 9/15/2015
7	Turtle, leatherback sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Unknown	1	1	Capture/Handle/Release	Net, Tangle	Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010 9/15/2015

# Location

Research Area: Atlantic Ocean State: FL Stream Name: Near-shore Sabellariid Worm Reefs, Indian River County Location Description: Study area extends from 27.8 latitude, -80.4 longitude to 27.7 latitude, -80.4 longitude.

### **Take Information**

Line Ve	r Species	Listing Unit/Stock	Production /Origin	Life Stage	Sex	Expected Take	Takes Per Animal	Take Action	Observe /Collect Method		Transport Record	Begin Date	End Date
1	Turtle, green sea	Range-wide (NMFS Threatened)	Wild	Adult/ Subadult/ Juvenile	Male and Female	140	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Lavage; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
2	Turtle, loggerhead sea	Range-wide (NMFS Threatened)	Wild	Adult/ Subadult/ Juvenile	Male and Female	10	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
3	Turtle, hawksbill sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Male and Female	2	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
4	Turtle, Kemp's ridley sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Male and Female	2	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015

# Location

Research Area: Atlantic Ocean State: FL Stream Name: Trident Turning Basin, Cape Canaveral Air Force Station, Cape Location Description: 28.4 latitude, -80.6 longitude

# **Take Information**

					Takes		Observe				
	Listing	<b>Production</b>		Expected	l Per		/Collect		<b>Transport</b>	Begin	
Line Ver Species	Unit/Stock	/Origin Life S	Stage Sex	Take	Animal	Take Action	Method	Procedure	Record	Date	<b>End Date</b>

1	Turtle, green sea	Florida Breeding Populations (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Male and Female	140	1	Capture/Handle/Release	Net, Tangle	Count/survey; Epibiota removal; Lavage; Mark, carapace (temporary); Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
2	Turtle, loggerhead sea	Range-wide (NMFS Threatened)	Wild	Adult/ Subadult/ Juvenile	Male and Female	10	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
3	Turtle, Kemp's ridley sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Male and Female	1	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
4	Turtle, hawksbill sea	Range-wide (NMFS Endangered)	Wild	Adult/ Subadult/ Juvenile	Male and Female	1	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, blood; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015
5	Turtle, leatherback sea	Range-wide (NMFS Endangered)	Wild	All except hatchling	Male and Female	1	1	Capture/Handle/Release	Net, Tangle	Epibiota removal; Mark, flipper tag; Mark, PIT tag; Measure; Photograph/Video; Sample, tissue; Weigh	N/A	9/3/2010	9/15/2015

### **NEPA Checklist**

1) If your activities will involve equipment (e.g., scientific instruments) or techniques that are new, untested, or otherwise have unknown or uncertain impacts on the biological or physical environment, please discuss the degree to which they are likely to be adopted by others for similar activities or applied more broadly.

No

2) If your activities involve collecting, handling, or transporting potentially infectious agents or pathogens (e.g., biological specimens such as live animals or blood), or using or transporting hazardous substances (e.g., toxic chemicals), provide a description of the protocols you will use to ensure public health and human safety are not adversely affected, such as by spread of zoonotic diseases or contamination of food or water supplies.

We will be collecting blood samples and tissue samples. Antiseptic protocol will be followed, including the use of sterile needles and biopsy punches, alcohol swabs, and the wearing of medical exam gloves.

3) Describe the physical characteristics of your project location, including whether you will be working in or near unique geographic areas such as state or National Marine Sanctuaries, Marine Protected Areas, Parks or Wilderness Areas, Wildlife Refuges, Wild and Scenic Rivers, designated Critical Habitat for endangered or threatened species, Essential Fish Habitat, etc. Discuss how your activities could impact the physical environment, such as by direct alteration of substrate during use of bottom trawls, setting nets, anchoring vessels or buoys, erecting blinds or other structures, or ingress and egress of researchers, and measures you will take to minimize these impacts.

Yes, the Pelican Island National Wildlife Refuge. There will be negligible alteration of the substrate by the anchors on either end of the tangle net.

4) Briefly describe important scientific, cultural, or historic resources (e.g., archeological resources, animals used for subsistence, sites listed in or eligible for listing in the National Register of Historic Places) in your project area and discuss measures you will take to ensure your work does not cause loss or destruction of such resources. If your activity will target marine mammals in Alaska or Washington, discuss measures you will take to ensure your project does not adversely affect the availability (e.g., distribution, abundance) or suitability (e.g., food safety) of these animals for subsistence uses.

No

5) Discuss whether your project involves activities known or suspected of introducing or spreading invasive species, intentionally or not, (e.g., transporting animals or tissues, discharging ballast water, use of equipment at multiple sites). Describe measures you would take to prevent the possible introduction or spread of non-indigenous or invasive species, including plants, animals, microbes, or other biological agents.

No

### **Project Contacts**

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Principal Investigator: Llewellyn Ehrhart

Other Personnel:

Name Role(s)

Dean Bagley Co-Investigator
Simona Ceriani Co-Investigator
William Edward Redfoot Co-Investigator
Brian Shamblin Co-Investigator
Michael Walsh Co-Investigator

#### **Attachments**

Application Archive - P14506T14Issued.pdf (Added Sep 28, 2010)

Contact - Brian Shamblin: C13528T5Shamblin CV.doc (Added Oct 6, 2009)

Contact - Dean Bagley: C7870T5BAGLEY VITAE 05-08[1].doc (Added Aug 4, 2009)

Contact - Llewellyn Ehrhart: C5469T5LME CV 02-05-09.doc (Added Aug 4, 2009)

Contact - Michael Walsh: C13027T5CV walsh 08 with grants and awards.doc (Added Apr 30, 2009)

Contact - Simona Ceriani: C13325T516598 Ceriani's CV\_ShortCV\_15\_Nov\_2011.doc (Added Feb 13, 2012)

Contact - William Edward Redfoot: C12996T5W Redfoot CV.doc (Added Aug 4, 2009)

Lit Review - P14506T7LITERATURE CITED NMFS permit renewal1.doc (Added Oct 6, 2009)

#### **Status**

Application Status: Application Complete
Date Submitted: December 9, 2009
Date Completed: December 10, 2009

FR Notice of Receipt Published: December 16, 2009 Number: 0648-xt27

Comment Period Closed: January 15, 2010 Comments Received: Yes Comments Addressed: Yes

Last Date Archived: March 8, 2012

• ESA Section 10(a)(1)(A) permit (other)

Current Status: Issued Status Date: September 2, 2010

Section 7 Consultation: Formal Consultation

NEPA Analysis: Environmental Assessment

Date Cleared by General Counsel: August 31, 2010

FR Notice of Issuance/Denial Published: October 4, 2010 Notice Number: 0648-xz15

Expire Date: September 15, 2015

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# **Modification Requests**

		Modification Requested				
Numbe	er Title	Description	Status	Date Submitted	Date Issued	Issued Version
1	Authorized Recipient for samples	Request to add Dr. Sarah Milton and Ms. Patricia Sposato-Ploski as authorized recipients of blood samples for analysisimmune function studies.	Issued (	02/22/2012	03/02/2012	

# Reports

# **Reports Required**

Nhr	Report Type	Report	Period	Data Dua	Status	Date Received
1101	Keport Type	<b>Start Date</b>	<b>End Date</b>	Date Due	Status	Date Received
1	Annual	09/03/2010	09/15/2011	12/15/2011	N/A	12/12/2011
2	Annual	09/16/2011	09/15/2012	12/15/2012	N/A	
3	Annual	09/16/2012	09/15/2013	12/15/2013	N/A	
4	Annual	09/16/2013	09/15/2014	12/15/2014	N/A	
5	Annual	09/16/2014	09/15/2015	12/15/2015	N/A	
6	Final	09/03/2010	09/15/2015	03/15/2016	N/A	